IN THE CLAIMS:

Please cancel claims 16, 20, 23 and 24 without prejudice.

Please add new claim 26 and 27.

Please amend claims 1, 2, 4-15, 17-19, 21, 22, and 25 as follows:

1. (Currently Amended) A process of preparing membrane vesicles from a biological sample, wherein said biological sample comprises membrane vesicles produced by antigen presenting cells that have been sensitized to one or more selected antigens, said method ~~characterised in that it comprises~~ comprising at least one anion exchange chromatography treatment of the sample.

2. (Currently Amended) Process according to claim 1, wherein said ~~characterised in that it comprises at least one strong~~ anion exchange chromatography is performed on a support functionalised witha quaternary amine ~~treatment step~~.

3. (Original) The method of claim 1, wherein the biological sample is a biological fluid, a culture supernatant, a cell lysate or a pre-purified solution.

4. (Currently Amended) Process according to claim 1, ~~any of the above claims, characterised in that~~ wherein the biological sample is selected from a biological fluid, a culture supernatant, a cell lysate and ~~or~~ a pre-purified solution.

5. (Currently Amended) A process of preparing membrane vesicles from a biological sample, wherein said process ~~characterised in that it~~ comprises at least:

a) the culture of a population of membrane vesicles producing antigen presenting cells under conditions enabling the release of vesicles, wherein said antigen presenting cells have been sensitized to one or more selected antigens,

11. (Currently Amended) A process of preparing membrane vesicles, wherein said process ~~characterised in that it~~ comprises the following steps :

a) the culture of a population of membrane vesicle ~~(e.g. exosome)~~ producing antigen presenting cells under conditions enabling the release of vesicles,

b) the treatment of the culture supernatant with at least one ~~ultrafiltration or~~ affinity chromatography step, to produce a biological sample enriched with membrane vesicles ~~(e.g. with exosomes)~~, and

c) an anion exchange chromatography ~~and/~~ or gel permeation chromatography treatment step of the biological sample.

12. (Currently Amended) Process according to claim 11, ~~characterised in that it~~ further comprisinges a sterilising filtration step d) of the treated preparation.

13. (Currently Amended) Process according to claim 1, wherein ~~any of the above claims characterised in that~~ the membrane vesicles have a diameter between approximately 60 and 90 nm.

14. (Currently Amended) Process according to claim 1, wherein ~~any of the above claims, characterised in that~~ the ~~membrane vesicles are vesicles produced by~~ antigen presenting cells comprise ~~, particularly~~ dendritic cells, B lymphocytes, macrophages or mastocytes.

15. (Currently Amended) Process according to claim 6, wherein ~~14, characterised in that~~ the membrane vesicles are vesicles produced by human dendritic cells~~, particularly of human origin~~.

16. (Cancelled)

17. (Currently Amended) A process of preparing membrane vesicles, wherein said process ~~characterised in that it~~ comprises the following steps:

a) obtaining a population of cells comprising immature dendritic cells,

b) culturing the dendritic cells under conditions enabling the production of membrane vesicles, and

c) purifying the membrane vesicles using a process comprising at least an anion exchange chromatography treatment.

18. (Currently Amended) A process of preparing membrane vesicles, wherein said process ~~characterised in that it~~ comprises the following steps:

a) obtaining a population of immature dendritic cells,

b) culturing the dendritic cells under conditions enabling the production of membrane vesicles,

c) treating the culture supernatant to produce a biological sample enriched with membrane vesicles~~, particularly by an ultrafiltration or affinity chromatography step~~, and

d) purifying the membrane vesicles using a process comprising at least an anion exchange ~~and/~~or gel permeation chromatography step.

19. (Currently Amended) Process according to claim 17, wherein ~~characterised in that~~ the dendritic cells are obtained from a biological sample from a subject~~, e.g. bone marrow or peripheral blood~~.

20. (Cancelled)

21. (Currently Amended) Process according to claim 17, wherein ~~any of claims 17 to 20, characterised in that~~ the dendritic cells are sensitised to an antigen, prior to the membrane vesicle production step b).

22. (Currently Amended) Process according to claim 17, wherein ~~[any of claims 17 to 21, characterised in that,]~~ during step b), the dendritic cells are cultured under conditions stimulating membrane vesicle production.

23-24. (Cancelled)

25. (Currently Amended) Composition comprising membrane vesicles prepared using the process according to claim 1 ~~any of claims 1 to 22~~.

26. (New) Process of preparing membrane vesicles from a biological sample, comprising:

a) the culture of a population of membrane vesicle producing tumoral cells under conditions enabling the release of vesicles,

b) a membrane vesicle enrichment step, and

c) an anion exchange chromatography or gel permeation chromatography treatment of the sample.

27. (New) Process according to claim 26, wherein the tumoral cells are human tumoral cells.

RESPONSE

The Amendments to the Claims Traverse All of the § 112 Issues.

Claims 1 and 5 have been amended to provide that the biological sample comprises membrane vesicles produced by antigen presenting cells that have been sensitized to one or more selected antigens. Support for this amendment can be found in the application, in particular page 18, lines 20-25 for instance. Furthermore, claim 5 has been amended to incorporate a step a), as requested by the Examiner.

Claim 6 is now independent and relates to a process of producing membrane vesicles from dendritic cells.

Claim 12 has been amended to recite a sterilising filtration step d).

Claims 2, 6, 7, 11, 14, 18, and 19 have been amended to overcome rejections made under 35 U.S.C. § 112.

In particular, the expression "strong anion exchange chromatography" has been replaced by "anion exchange chromatography performed on a support functionalised with a quaternary amine." Support for this amendment can be found for instance at page 7 (lines 7-9).

The expression "preferred" or in parentheses have been removed from the quoted claims. Furthermore, claim 11 now relates to a process using antigen presenting cells and claim 18 (as claim 17) now refers to immature dendritic cells.

New claims 26 and 27 have been added, related to a process using tumoral cells. These claims are based on previous claim 16, now deleted.

These amendments find explicit support in the specification as filed, do not add any new matter, and entry thereof is respectfully requested. It is believed that the proposed amendments overcome the claim objections and rejections under 35 U.S.C. 112, as well as under 35 U.S.C. 101.

THE ART-BASED REJECTIONS UNDER 35 U.S.C. § 102 ARE TRAVERSED – THE CLAIMS RECITE AN ELEMENT NOT DISCLOSED BY THE REFERENCES.

Numerous prior art references have been cited against claims 1, 2, 3, 5, 7, 11, 12 and 23 of the application under 35 U.S.C. § 102(b). Claim 23 is deleted. None of these references disclose the step of culturing a population of membrane vesicle producing antigen-sensitized, antigen-presenting cells (claims 5 and 7) nor the culture of antigen-presenting cells, and the specific affinity and avian exchange/gel permeation. The following headings correspond to the paragraphs of the Office Action.

9. Michaelson et al Does Not Anticipate Amended Claims 5 and 7.

Michaelson et al. and Alberts et al. disclose methods of purifying synaptic vesicles. Without reaching the issue of whether the purified synaptic vesicles of Michaelson are equivalent to the membrane vesicles of the present invention, Michaelson et al. does not disclose culturing antigen-sensitized, antigen-presenting cells from the population of membrane vesicles (claim 5) and does not disclose the clarifying stage as part of an enrichment step for these cells (claim 7).

10. Dubinsky Does Not Disclose the Step of Culturing a Population of Membrane Vesicles Producing Sensitized Antigen-Presenting Cells.

Dubinsky et al. disclose the preparation of apical membrane vesicles from bovine tracheal epithelium. No antigen sensitization is disclosed or even contemplated by Dubinsky et al. – claims 5 and 7 are not anticipated by the reference.

11. Von der Decken Does Not Disclose the Step of Culturing a Population of Membrane Vesicles Producing Antigen-Sensitized Antigen-Presenting Cells.

In Von der Decken, microsomes are prepared from pellet consisting of ribosomes derived from homogenized liver. Tanaka, Gordon, Smith, Seeger and Nishino do not describe the origin of the membrane shed-vesicles mentioned. None of them suggests that membrane vesicles are produced by antigen presenting cells. Chen et al. teach that polyunsaturated acid-containing molecular species of phosphatidylserine can be isolated from bovine extract by means of anion exchange chromatography but do not suggest the possibility of purifying vesicles produced by antigen presenting cells by such a chromatography step. Olge et al. teaches affinity chromatography, but not applied to vesicles produced by antigen-presenting cells.

Thus, the claims recite elements not disclosed by the cited references and the pending claims cannot be rendered unpatentable under § 102 by these references.

12 and 13.: Claims 1, 2, 5, and 7 are Not Anticipated By Vaandrager et al. or Denning et al.

Vaandrager et al. describes vesicles derived from villi cells and Denning et al., microsomal pellet derived from C. reinhardtii cells. There is no disclosure of culturing antigen-presenting cells that have been sensitized to an antigen.

14. Feldman et al. Does Not Disclose Antigen-Sensitized, Antigen-Presenting Cells.

Feldman et al. describes the purification of the human erythroid BPA protein from lymphocytes plasma comprises or that shed into plasma membrane-derived LCM vesicles. The purification describes the extraction of BPA from the membrane with reagents (NaOH, octyl glycoside, etc.) followed by bel or ion exchange chromatography. Hence, the authors are not

purifying vesicles, but instead a protein component. Furthermore, these extraction techniques would destroy the intactness (physical shape) of exosomes according to the invention. The elutoin of the BPA-fraction to the ion exchange column (DE-52; DEAE-cellulose) requires 0.2 M NaCl, exosomes being eluted in the invention at a much higher NaCl concentration (see for example pages 37 and 38 of the specification). Feldman et al. thus does not describe size exclusion or ion exchange chromatographic purification of exosomes and also does not describe the origin of the membrane shed-vesicles mentioned.

15-18.: The References of Record Do Not Establish a Prime Facie Case under § 103(a)

Unlike any of the cited references, taken alone or in combination, the present invention advantageously allows the high purification of membrane vesicles, particularly of vesicles which include heterologous molecules (antigenic determinants or epitopes for example) without damaging said heterologous molecules. It is particularly striking and unexpected that the integrity and functionality of complex and sensitive molecular complexes such as MHC-peptides complexes, can be preserved in such chromatographic techniques. Purified vesicles of the invention thus retain their biological properties, i.e., their ability to present their heterologous molecules or transmit them to antigen presenting cells. The references not addressed above, Zitvogel et al.. Raposo et al., and Thiery et al. relate to dexosomes but do not teach anion exchange chromatography. Others describe methods of purifying vesicles but do not teach the production of membrane vesicles from antigen presenting cells.

While the references disclose different techniques and methods for manipulating vesicles, Applicants submit that these nearly two dozen references do not, when taken alone or in combination, disclose each and every limitation of the claimed methods. Applicants further submit

that no motivation to combine these references in the manner suggested exists in the prior art. Thus, the cited combination of references does not create a *prima facia* case of obviousness, and applicants request that the rejection of claims 1-3, 5-7, 11, 12, 17-19, and 23-24 be withdrawn in light of these comments.

Applicants respectfully note that to establish a *prima facia* case of obviousness, all elements of the claimed invention must be shown in a combination of the prior art and some motivation to combine these elements in the claimed manner must be explicitly demonstrated. Applicants submit that such motivation cannot be demonstrated here because the references do not fairly disclose each element of the claims when taken as a whole, even if isolated reference to individual elements is present. As recently restated by the Federal Circuit in In re Werner Kotzab, 55 USPQ2d 1313 (June 30, 2000):

> Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant. [C.O.]. . .
>
> ....
>
> While the test for establishing an implicit teaching, motivation, or suggestion is what the combination of these two statements of Evans [the reference] would have suggested to those of ordinary skill in the art, the two statements cannot be viewed in the abstract. Rather, they must be considered in the context of the teaching of the entire reference. Further, a rejection cannot be predicated in the mere identification in Evans of individual components of the claimed inventions. Rather, particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.

(C.O. indicates citation omitted, emphasis and parenthetical statements added)

Two well established principles of the law of obviousness under 35 U.S.C. § 103 are "(B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination" and "(C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention," See MPEP § 2141, Basic Considerations Which Apply to Obviousness Rejections. Springing from these requirements are two other rules regarding the interpretation of prior art references: (1) as first explained in In re Gordon, 733 F.2d 900 (Fed. Cir. 1984), a prior art reference may not be modified in a way that would render the prior art invention unsatisfactory for its intended purpose (See MPEP § 2143.01), and (2) A prior art reference describing a composition of matter with similar parameters will only render a claimed similar composition obvious by "optimization" of the parameters if the parameter was art recognized as result-effective. In Re Antonie 195 USPQ 6 (CCPA 1977) (See MPEP § 2144.05, II. B.).

Because the cited references do not establish a prima facie case under § 103, and because no combination or modification of the cited references can foreclose patentability of the pending claims, Applicants submit that the pending claims are in condition for allowance and request such action accordingly.

Respectfully submitted,

ORRICK, HERRINGTON & SUTCLIFFE LLP

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By: _____
Kurt T. Mulville, Reg. No. 37,194

4 Park Plaza, Suite 1600
Irvine, CA 92614
949/567-6700 X 7740 Telephone
949/567-6710 Facsimile